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Research Article

Evaluation of the effects of artemisia annua L. essential oil on the physicochemical, structural, and antimicrobial properties of fish gelatin-based edible films

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Abstract

In the present study, a novel antimicrobial film was developed using fish gelatin (FG), carboxymethylcellulose (CMC), and Persian gum (PG) incorporated with Artemisia annua (AEO) essential oil at three different concentrations (0.5%, 1%, and 1.5%, w/v). The films were fabricated using the casting method, and their physicochemical, structural, and antimicrobial properties were thoroughly evaluated. The results indicated that as the AEO content increased, the water vapor permeability (WVP) of the films, ranging from 0.746 to 4.51 g mm/kPa h m², and the solubility (FS) of the films both increased, while the water absorption rate (SR) decreased. Mechanical property analysis revealed that the incorporation of AEO significantly enhanced the flexibility (EAB) of the films ($P<0.05$), while reducing the tensile strength (TS). FT-IR spectroscopy revealed the formation of intermolecular hydrogen bonds between the functional groups of the polymer components and AEO, which contributed to the enhanced thermal stability and mechanical properties of the ternary films. X-ray diffraction (XRD) analysis further demonstrated the miscibility of the film components. Scanning electron microscopy (SEM) imaging confirmed that the ternary films containing AEO exhibited a continuous, smooth, and homogeneous microstructure, indicating good compatibility between the components. In addition, the ternary films containing the highest concentration of AEO (1.5%) showed acceptable antibacterial activity against the Gram-positive bacteria *Staphylococcus aureus* and *Listeria monocytogenes* and the Gram-negative bacteria *Salmonella enteritidis* and *Escherichia coli*. The findings of this study indicate that ternary FG/CMC/PG films containing AEO can be used as promising packaging materials for food products.

Keywords: Antimicrobial films, Fish gelatin, Carboxymethyl cellulose, Persian gum, Artemisia annua L. essential oil

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1. Introduction

The first interaction between the customer and a product occurs through its packaging, which largely serves to present a favorable image of the product [1]. Nowadays, new food packaging technologies are being developed in response to consumer demands and the food industry's trend toward producing fresh products with extended shelf life and controlled quality [2]. Moreover, changes in retail practices and consumer lifestyles have posed significant challenges to the food packaging industry and have acted as a driving force for the development of new and improved packaging concepts aimed at extending shelf life and ensuring the quality and safety of food products [3]. Active packaging is one of the new concepts in food packaging that has emerged as a response to the continuous changes in recent consumer needs and market trends. Active packaging is an innovative approach aimed at preserving or extending the shelf life of food products while ensuring their quality, safety, and integrity. As defined in European Regulation (EC) No. 450/2009, active packaging includes packaging systems that interact with food in such a way that they "deliberately incorporate components that release or absorb substances into or from the packaged food or the food environment" [4]. Furthermore, the growing trend of "green consumerism" has increased consumer pressure on industries to replace synthetic antimicrobial agents with natural alternatives (such as plant extracts or essential oils) in order to ensure food safety [5].

Essential oils (EOs) are a group of antimicrobial substances that are "generally recognized as safe" (GRAS) and acknowledged by the U.S. Food and Drug Administration (FDA) [6]. The Artemisia plant, belonging to the Asteraceae family, comprises over 400 species worldwide, with 34 species widely distributed in Iran. This plant is known by various names in Iran, including *Khargoosh*, *Caspian wormwood* (*Artemisia annua L.*), and *sweet wormwood*, and is extensively grown in the northern regions of the country [7]. The essential oils extracted from various parts of this plant are used in the food, cosmetic, hygienic, and pharmaceutical industries, and possess antifungal, antibacterial, antiviral, and antitumor properties [8]. The major significance of this plant lies in the presence of a sesquiterpene lactone endoperoxide compound called artemisinin, which serves as a key compound for the production of pharmaceutical drugs [8]. Natural additives such as essential oils can be incorporated into active packaging (in the form of films and coatings) to enhance physicomechanical properties and impart antimicrobial activity [4]. For example, in a study by Hosseini et al. [5], cinnamaldehyde essential oil was added to a chitosan-based edible film to improve its physical properties and induce antimicrobial effects. The results confirmed that the addition of essential oil led to a reduction in the film's solubility and water absorption by approximately 47% and 87%, respectively. Moreover, the edible film containing 1.6% (w/v) cinnamaldehyde effectively inhibited the growth of food spoilage microorganisms, creating an inhibition zone ranging from approximately 15.4 to 19 mm.

On the other hand, the growing environmental awareness regarding the harmful effects of plastic packaging materials, along with efforts to reduce waste volume, has led to an increased use of packaging materials based on biopolymers derived from renewable sources. Food-grade biopolymers such as proteins (e.g., fish gelatin, FG) have been successfully incorporated into packaging films due to their unique functional properties, including flexibility, transparency, and superior barrier properties against oxygen and ultraviolet radiation [9]. Fish gelatin, due to its lower content of proline and hydroxyproline in its structure, exhibits better moisture barrier

properties compared to other types of gelatin. However, the overall moisture barrier performance of gelatin-based films is still weaker than that of other polymers [9]. In this regard, one of the effective approaches to overcoming these limitations is the combination of two or more polymers to form composite films [10]. Carboxymethyl cellulose (CMC), a derivative of cellulose obtained by substituting some hydroxyl groups with carboxymethyl groups, is a biopolymer known for its excellent film-forming properties, low cost, thermoplastic behavior, and resistance to biodegradation. Therefore, combining it with FG (fish gelatin) can result in a biocomposite with improved physical properties [11].

Nowadays, the use of gums for the preparation of composite films has attracted significant attention from researchers, primarily due to their potential to reduce production costs on a commercial scale and enhance certain properties of biocomposites. Gums are a group of high molecular weight polysaccharides capable of stabilizing emulsion systems and are derived from plant exudates and marine algae [12]. As hydrocolloids, gums possess the ability to form films [13]. Persian gum (PG), also known as "Zedo gum" or "Angum gum," is a carbohydrate polymer exuded from the bark of wild almond trees (*Amygdalus scoparia* Spach), which are predominantly found in Iran [12]. As an arabinogalactan, PG can function as a fat replacer, stabilizer, emulsifier, gelling agent, and film-forming agent [14]. In this regard, a biocomposite film based on wheat gluten containing Persian gum (PG) and guar gum (GG) was prepared and its physicochemical properties were investigated by Najafian et al. [15]. The results of FTIR and SEM analyses confirmed that wheat gluten exhibited good miscibility with PG and GG. Furthermore, increasing the concentration of gums in the film-forming solution led to a reduction in film thickness and improvement in solubility and water vapor permeability (WVP) of the films. Based on the information presented, no study has yet been conducted on the preparation of a three-component biocomposite film based on fish gelatin/carboxymethyl cellulose/Persian gum (FG/CMC/PG) containing AEO. Therefore, the main objective of this study is to prepare FG/CMC/PG biocomposite films containing different concentrations of AEO (0.5%, 1%, and 1.5%) and to evaluate the physical, mechanical, structural, thermal, and antimicrobial properties of the resulting films for food packaging applications.

2. Materials and Methods

2.1. Materials

Fish gelatin (FG) obtained from the skin of cold-water fish (Bloom strength 240) and carboxymethyl cellulose (CMC) (molecular weight 250,000 g/mol, high viscosity, CP 3000-1500) were purchased from Sigma-Aldrich (USA). Acetic acid, glycerol, filter paper (Whatman 42), Tween 80, tryptic soy broth (TSB), and plate count agar (PCA) were obtained from Merck (Germany). Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923) and *Listeria monocytogenes* (ATCC 13932), as well as gram-negative bacteria *Salmonella enteritidis* (ATCC 18034) and *Escherichia coli* (ATCC 25922), were obtained from the Pasteur Institute of Amol.

2.2. Preparation of Ternary Packaging Films of Fish Gelatin/Carboxymethyl Cellulose/Persian Gum Containing Caspian Wormwood Essential Oil

Ternary films of fish gelatin/carboxymethyl cellulose/Persian gum with a ratio of (54FG/36CMC/10PG) were prepared using the casting method. To produce the FG/CMC/PG film, 3% (w/v) FG, 1.5% (w/v) CMC, and 3% (w/v) Persian gum solutions were first prepared. After preparing the solutions, the FG and CMC

solutions were first mixed and placed on a magnetic stirrer at 45°C for 45 minutes [16]. After mixing, the PG film solution (at a 10% level) was gradually added to the FG/CMC solution, and the resulting mixture was stirred on the magnetic stirrer at the same temperature for an additional 45 minutes. Then, glycerol (0.3 g per gram of dry matter) and Tween 80 (at a ratio of 1:5, Tween to Caspian wormwood essential oil, AEO) were added to the solution and stirred for another 30 minutes at the same temperature. Subsequently, AEO was added to the mixture at three levels (0.5%, 1%, and 1.5%) and stirred using a homogenizer at 13,000 rpm for 10 minutes. The resulting film-forming solution was then placed in a vacuum oven for 45 minutes to remove air bubbles. After degassing, the solution was gently poured into plastic plates and placed in an oven at 45°C for 48 hours to dry.

2.3. Evaluation of Physical and Mechanical Properties of the Produced Films

2.3.1. Film Thickness

The thickness of the samples was measured using a digital micrometer (accuracy of 0.0001 mm, Mitutoyo, Japan). Measurements were randomly taken at nine different points on each sample. The average thickness of these points was used to determine the physical and mechanical properties of the films.

2.3.2. Water Vapor Permeability (WVP) Measurement

The WVP test was carried out according to the standard ASTM E96/E96M-05 method [17]. For this test, the produced films were sealed onto the opening of a glass cup (diameter: 49 mm) containing 6 mL of distilled water (100% relative humidity, water vapor pressure: 2.337×10^3 Pa at 20°C) using silicone grease. The edges of the cups were gently sealed with parafilm. The sealed and weighed cups were then placed in a desiccator containing silica gel at room temperature. The weight of the cups was recorded at 2-hour intervals over a 12-hour period. Finally, the water vapor permeability (WVP) of the films was calculated using Equation (1).

$$WVP = \frac{WVTR \times L}{\Delta P} \quad (1)$$

In this equation, WVP represents the water vapor permeability expressed in g·mm/KPa·h·m², WVTR is the water vapor transmission rate, L is the average thickness of the film (mm), and ΔP is the difference in vapor pressure between the inside and outside of the cells.

2.3.3. Water Solubility Measurement

Initially, film samples with dimensions (40×10 mm) were placed in an oven at 105°C for 24 hours to determine their initial dry weight. After weighing, the samples were transferred to containers containing 30 mL of distilled water and left at room temperature for 24 hours. The samples were then passed through pre-weighed filter paper and placed along with the filter paper in a glass plate, which was placed in the oven at 105°C for an additional 24 hours. After drying, the samples were weighed again, and the water solubility of the films was calculated using Equation (2) [5].

$$\text{Solubility Percentage} = 100 \times (\text{Film Weight after Immersion} - \text{Initial Dry Film Weight}) / \text{Initial Dry Film Weight} \quad (2)$$

2.3.4. Contact Angle Measurement of the Films

To measure the contact angle of the films, the sessile drop method was used. In this method, 5 microliters of deionized water were placed on the samples twice using a PG-X goniometer (Switzerland), and the contact angle of the drop with the film was reported at the initial time [5].

2.3.5. Measurement of Mechanical Properties

The tensile strength (TS) and elongation at break (EAB) of the films were measured using a universal testing machine model TVT-300Xp (Sweden) following the standard ASTM D882-02 testing method [18]. The film samples were first cut into rectangular shapes with dimensions of 10×60 mm and placed in a desiccator containing silica gel to achieve moisture equilibrium at 25°C for 24 hours. Then, the samples were placed between the two grips of the testing machine. The initial distance between the grips and the speed of the upper grip were set to 30 mm and 50 mm/min, respectively.

2.3.6. Measurement of Surface Color of the Films

The color of the films was determined using a colorimeter model BYK Gardner (USA). Initially, the film samples were placed on a standard white background. Then, the values of L*, a*, and b*, representing luminosity (L*), greenness-redness (a*), and blueness-yellowness (b*), respectively, were measured for each sample using the colorimeter. Each measurement was repeated three times, with readings taken randomly at three different points in each replicate. The total color difference (ΔE) was calculated using Equation (3), after subtracting the differences between the L*, a*, and b* indices of the sample and those of the standard white background (L* = 94.63, a* = -0.88, b* = 0.65).

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2} \qquad (3)$$

2.4. Evaluation of Structural Properties of the Produced Films

2.4.1. Fourier-Transform Infrared Spectroscopy (FTIR)

Fourier-transform infrared (FTIR) spectroscopy in transmission mode was performed using an FTIR spectrophotometer (Perkin-Elmer, Shelton, CT, USA). Prior to the test, the film samples were dried in a desiccator containing silica gel for two weeks. Then, film pieces with a diameter of 2 cm were placed between two KBr plates. The spectra were recorded in the range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1}.

2.4.2. X-ray Diffraction (XRD) Test

The crystalline structure of the prepared films with a thickness of 0.02 mm was analyzed using an X-ray diffraction (XRD) device (Siemens D5000) over a 2θ range of 2–50 degrees, with a scanning rate of 0.02 degrees per minute.

2.4.3. Differential Scanning Calorimetry (DSC) Analysis

To investigate the thermal behavior of the prepared films, a Differential Scanning Calorimeter (DSC) model DSC-200 F3 (NETZSCH, Germany) was used. Approximately 10 mg of each sample, which had been stored in a desiccator containing silica gel for two weeks, was placed in aluminum pans. The samples were scanned at an approximate rate of 10 °C/min. The thermal cycle for each sample was performed within a temperature range of 25 to 400 °C under a constant nitrogen atmosphere. From the resulting thermal curves, the glass transition temperature (Tg, °C), melting point (Tm, °C), and enthalpy (ΔH, J/g) associated with the melting processes were determined.

2.5. Scanning Electron Microscopy (SEM) Analysis

To investigate the microstructures and to observe the surface and cross-sectional morphology of the prepared films, scanning electron microscopy (SEM) was performed using a SEM device (XL30 ESEM, Philips, Netherlands) operated at 20 kV. For cross-sectional imaging, the films were immersed in liquid nitrogen and manually fractured. The samples were then

mounted on aluminum stubs using double-sided adhesive tape and coated with a thin layer of gold prior to imaging.

2.6. Evaluation of Antimicrobial Properties of the Prepared Films

To assess the antimicrobial properties of the fabricated films, the direct contact method was employed [19]. For this purpose, sterilized film samples with a diameter of 1.5 cm were prepared and placed on tryptone soy agar (TSA) plates inoculated with 10^6 CFU of bacteria. Subsequently, all plates were incubated at 37°C for 24 hours. At this stage, bacterial growth was evaluated by measuring the diameter of the inhibition zone (in mm). To ensure uniform bacterial growth on the plate surfaces, a control plate without any film was included for each tested bacterium. Additionally, a plate without bacterial inoculation was used to confirm the sterility of the culture media.

2.7. Statistical Analysis

The experiment was conducted using a completely randomized design, and the statistical analysis of the data was performed using SPSS software version 23. Initially, the normality of the data was assessed using the Kolmogorov-Smirnov test, followed by the homogeneity of variances, which was tested using Levene's test. To determine the differences between treatments, a one-way analysis of variance (ANOVA) was used. In cases where the overall treatment effect was significant, Duncan's multiple range test was employed for mean comparisons. The significance level for rejecting H0 was set at 5% in all stages of the statistical analysis.

3. Results and Discussion

3.1. Water Vapor Permeability (WVP)

One of the important applications of food packaging is to reduce the transfer of moisture between the surrounding environment and the packaged food, which helps maintain the quality of the packaged food. Since the high permeability of biodegradable films to water vapor is not desirable for packaging applications, the raw materials selected for film production should therefore have a minimal Water Vapor Permeability (WVP) [20]. As shown in Table 1, the WVP value of the control film (FG/CMC/PG) was 0.746 ± 0.018 g·mm/kPa·h·m², and the addition of Artemisia essoil (AEO) at concentrations ranging from 0.5% to 1.5% to the polymer matrix significantly increased the WVP of the films (ranging from 2.71 ± 0.02 to 4.51 ± 0.05 g·mm/kPa·h·m²). A similar trend was observed in fish gelatin films containing bergamot essential oil [21], as well as in chitosan-based films containing basil essential oil [22]. According to Bonilla et al. [22], this result can be attributed to the discontinuities formed in the polymer network by the oil droplets, which lead to a loss of film integrity and, consequently, an increase in water vapor transmission through the film. In addition, the hydrophilic–hydrophobic balance of the film components directly affects the water vapor transmission process through the film. The increase in WVP may also be related to the increased thickness of the films, which is consistent with the findings of Kao and Song [23]; these researchers demonstrated that the WVP of loquat seed starch films containing oregano essential oil increases with the thickness of the film layer. However, other studies on the incorporation of essential oils into biopolymer matrices have reported a decrease in WVP [24, 25]. Therefore, it cannot be easily assumed that the WVP of edible films will necessarily decrease with the addition of a hydrophobic

component to the film matrix, although the effect of lipid incorporation on the structure of emulsified films is a determining factor in their water vapor barrier properties [26].

Table 1. Thickness, water vapor permeability (WVP), film solubility (FS), swelling ratio (SR), tensile strength (TS) and elongation-at-break (%EAB) values of FG/CMC/PG ternary films containing different amounts of AEO.

Sample	Thickness (mm)	Water Vapor Permeability (g mm/kPa h m2) WVP	Film solubility (%) FS	Water Absorption (%) SR	Tensile Strength (MPa) TS	Elongation at break (%) EAB
Control	0.06±0.00^{a}	0.75±0.02^{c}	37.04±1.04^{c}	219.30±12.29^{a}	16.33±3.97^{a}	39.10±3.60^{c}
0.5% AEO	0.08±0.00^{a}	2.71±0.02^{b}	40.23±2.97^{b}	207.33±6.36^{b}	11.38±2.02^{b}	79.58±3.18^{b}
1% AEO	0.11±0.01^{a}	4.20±0.29^{a}	40.77±3.46^{b}	182.99±10.21^{c}	9.32±2.55^{b}	90.46±4.52^{a}
1.5% AEO	0.12±0.01^{a}	4.51±0.05^{a}	53.48±0.91^{a}	158.22±6.51^{d}	5.41±1.64^{c}	93.25±3.73^{a}

The means in each column with different lowercase English letters are significantly different from each other ($P < 0.05$).

The data are presented as mean ± SD.

3.2. Film Solubility

Water insolubility or resistance is one of the important characteristics of edible films for packaging and food protection applications, especially in cases where water activity is high [5]. As shown in Table 1, the water solubility of the control FG/CMC/PG film was approximately 37%, which is lower than the values reported by Fernández et al. [27] for composite films based on carboxymethyl cellulose and gelatin (around 48%). The lower solubility of the bio-composite films in the present study may be attributed to protein–polysaccharide interactions (mainly through hydrogen bonding), which play a significant role in film formation [28]. However, differences between studies may also be due to variations in polymer concentration and different film preparation methods, which affect the final properties of the film [26]. With the addition of AEO to the film, solubility increased significantly (Table 1) (P<0.05), which may be attributed to the interactions between the protein–polysaccharide matrix and the essential oil, leading to a weakening of the interactions that stabilize the protein–polysaccharide network structure [26]. The presence of essential oil may also limit the interaction of glycerol with the protein matrix and hinder the formation of a continuous film network [29]. Similarly, in the study by Gómez-Estaca et al. [28], the addition of clove essential oil to a gelatin–chitosan film matrix resulted in an increase in the film's water solubility.

3.3. Swelling Ratio (SR)

Swelling behavior is also important for designing packaging materials and predicting stability and quality changes that may occur during storage [5]. As shown in Table 1, the control film FG/CMC/PG exhibited the highest SR (219.30 ± 12.29%), which may be attributed to the hydrophilic nature of the polymers and, in particular, the high water absorption capacity of gelatin (due to the presence of polar peptides) [30]. As shown in Table 1, the addition of varying levels (0.5% to 1.5%) of AEO, with its hydrophobic nature, significantly reduced the SR of the film (ranging from 207.33 ± 6.36% to 158.22 ± 6.51%) (P<0.05), which can be attributed to the presence of phenolic groups in the film composition [31]. Furthermore, the presence of AEO fills the internal cavities between the polymer chains, thus further reducing the water absorption of the films [5]. Additionally, the essential oil molecules may

form hydrogen bonds with the FG/CMC/PG molecules, thereby blocking the free hydroxyl groups in these polymers and leading to a reduction in the water absorption of the film [32]. Shahbazi [33] reported that the addition of grape seed extract reduced the water absorption of gelatin films from 34.43% to 23.82%.

3.4. Mechanical Properties of Films

Mechanical properties are important factors in selecting the type of packaging for food applications, and having quantitative information about the mechanical indices (tensile strength (TS) and elongation at break (EAB)) of biodegradable films is crucial for designing packaging materials and predicting their ability to maintain integrity during use as packaging material [34]. The TS and EAB results of the prepared films are shown in Table 1. The TS of the control composite film (FG/CMC/PG) was approximately 16.3 MPa, which is similar to the values reported by He et al. [35] for carboxymethyl cellulose/gelatin composite films (16.6 MPa). With the addition of AEO from 0.5% to 1.5%, the TS of the film significantly decreased from 16.33 ± 3.97 MPa to 5.41 ± 1.64 MPa (at 1.5% essential oil concentration), which is likely due to the structural discontinuities caused by the integration of the essential oil, affecting the mechanical behavior of the film (Sánchez-González et al., 2009). Sánchez-González et al. [36] also reported that the addition of tea tree oil (3-5%) significantly reduced the TS values of hydroxypropylmethylcellulose (HPMC) films. With the addition of AEO, the EAB of the bio-composite films increased from 39.10% (control film) to 93.25% for films containing 1.5% essential oil ($P<0.05$), which is consistent with the results reported by other researchers when incorporating plant essential oils into edible film matrices [24, 37]. The increase in flexibility may be attributed to the plasticizing role of the essential oils in the polymer matrix, which leads to improved formability properties [38]. Additionally, the incorporation of lipids or oils into protein- or polysaccharide-based films can interfere with the polymer chain-to-chain interactions and create flexible domains within the film [25].

3.5. Surface Color

The color of biodegradable films is of great importance in their application in the packaging industry and is considered one of the determining factors of the quality of the prepared film [5]. In general, the more transparent a polymer film is and the more it resembles synthetic plastics, the higher its acceptance and application will be, as the appearance characteristics of the packaged product will be fully visible. The values of L*, a*, b*, ΔE, WI, and opacity of the FG/CMC/PG films containing different levels of AEO are shown in Table 2. The appearance color of the film samples, determined by Hunter color values, shows that the ΔE values of the control film decreased slightly after the addition of AEO, mainly due to the increase in L* values and the decrease in a* and b* values. As mentioned, the redness (a*) of the prepared films significantly decreased from 4.74 ± 0.17 (control film) to 4.04 ± 0.17 (film containing 1.5% AEO); this characteristic may help prevent oxidative light degradation of the packaged foods [39]. The whiteness index (WI) in the composite films also increased with the higher percentage of essential oil (Table 2). Regarding opacity, the value obtained for the three-component FG/CMC/PG film was 1.40 ± 0.09 (Table 2). As shown in Table 2, the addition of AEO increased the opacity of the resulting films, reaching 7.14 ± 0.15 AU/mm at a 1.5% essential oil ratio. The increase in opacity of the films containing AEO can be attributed to the scattering of light by the oil droplets (with a different refractive index) within the polymer network of the film, which interferes with light transmission [40]. Similar results were reported by Ahmad et al. [39] regarding the effect of pomegranate peel extract on carboxymethyl cellulose-gelatin composite films.

Table 2. Colour parameters of of FG/CMC/PG ternary films containing different amounts of AEO

Sample	Colour parameters					Opacity
	L^*	α^*	b^*	ΔE	WI	
Control	24.43±0.72^b	4.74±0.17^a	10.40±0.32^a	71.09±0.68^a	23.56±0.69^b	1.40±0.09^c
0.5% AEO	28.20±0.39^a	4.34±0.14^b	8.63±0.29^b	67.10±0.35^b	27.55±0.36^a	2.83±0.05^c
1% AEO	28.48±1.21^a	4.17±0.23^b	8.76±0.59^b	66.82±1.18^b	27.82±1.18^a	4.01±0.18^b
1.5% AEO	29.70±0.82^a	4.04±0.17^b	8.25±0.17^b	65.56±0.83^b	29.09±0.83^a	7.14±0.15^a

The means in each column with different lowercase English letters are significantly different from each other ($P < 0.05$).
The data are presented as mean ± SD.

3.6. Fourier Transform Infrared (FTIR) Spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy is used to examine the functional groups, types of bonds, and interactions occurring in polymer blends [5]. The FTIR test results for the various produced films are shown in Figure 1. In the spectrum of the control film (FG/CMC/PG), a strong peak at 3364 cm-1 corresponds to the stretching vibrations of O-H and N-H groups, which typically indicate hydrogen bonding and molecular interactions [41]. This result is likely attributed to intermolecular interactions between the hydroxyl group of CMC and the carboxyl group of FG [42]. Two other strong peaks at 2888 cm-1 and 1641 cm-1 are related to the asymmetric stretching of C-H and the CH2 group (Amide III), respectively [43]. Additionally, the peaks observed at 1553, 1411, and 1154 cm-1 can be attributed to the solid carboxylate group -C=O and -COO, C-H bending, and possibly CN or NH bending, respectively [42]. The peaks at 1095 and 899 cm-1 are also assigned to C-O stretching vibrations [44]. When AEO (at levels of 0.5% and 1.5%) was added to the three-component film FG/CMC/PG, noticeable changes in the peak wavelengths were observed (Figure 1). For example, the O-H peak shifted from a wavelength of 3364 cm-1 in the control film to wavelengths of 3349 and 3350 cm-1 in the films containing 0.5% and 1.5% AEO, respectively, indicating the formation of hydrogen bonds between the essence and the FG/CMC/PG film matrix [45]. Additionally, after adding AEO, the 1641 cm-1 peak in the control film shifted to lower wavelengths (1636/1637 cm-1), which can be attributed to changes in the molecular organization of the film matrix, particularly its interaction with the gelatin polypeptide chains [39]. Furthermore, the Amide II peak shifted from 1553 cm-1 to 1546/1545 cm-1, indicating the presence of hydrogen bonds and hydrophobic interactions between AEO and the FG/CMC/PG film matrix [45]. Overall, the FTIR results suggest that the incorporation of AEO may have altered the molecular structure of the three-component film.

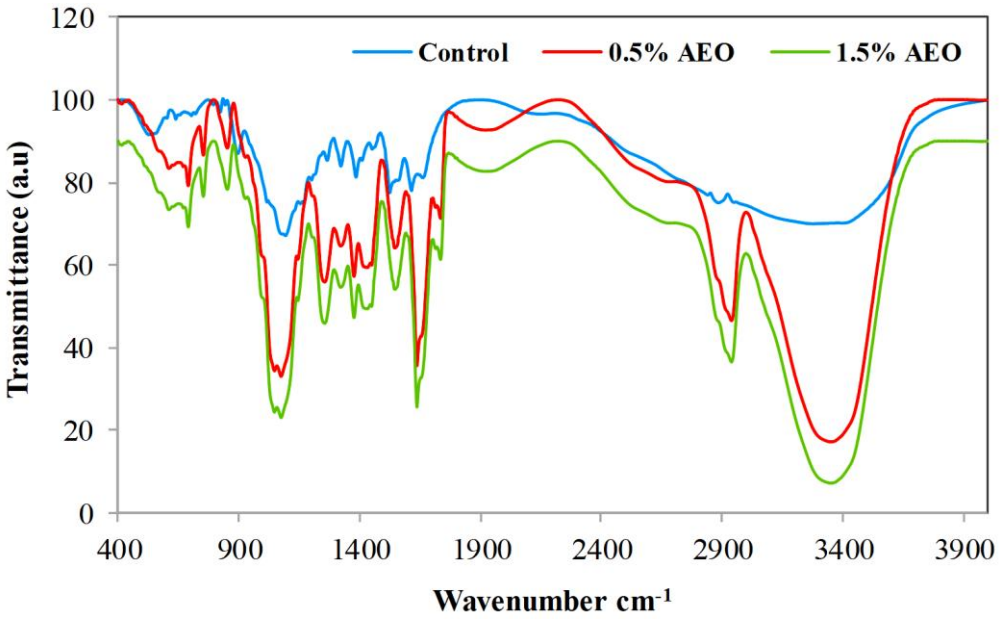

Fig 1. FTIR spectroscopy of control and FG/CMC/PG ternary films containing different amounts of AEO.

3.7. X-ray Diffraction (XRD)

XRD analysis was performed to investigate and evaluate the potential changes in the crystallinity of the three-component films due to the addition of AEO (Figure 2). As shown in Figure (2), the three-component control film (FG/CMC/PG) exhibited two distinct peaks at 9.92° (related to the triple-helix crystalline structure of gelatin) and 23.58° (characteristic of an amorphous and disordered phase), indicating the semicrystalline nature of the polymers [46]. With the addition of 0.5% and 1% AEO to the three-component films, only a single distinct peak was observed at 23.65° and 23.53° 2Θ, respectively. This suggests that the interaction between AEO and the film matrix leads to molecular reorganization, consequently altering the polymeric molecular structure [45]. Additionally, the presence of a single, distinct diffraction peak in the X-ray diffraction pattern of the three-component film containing AEO indicates good compatibility between the film components. This compatibility is likely due to hydrogen bonding and electrostatic interactions between the components, which lead to a reduction in recrystallization [47]. Similar results were observed by Nguyen et al. [42] when adding shallot extract to gelatin/guar gum films.

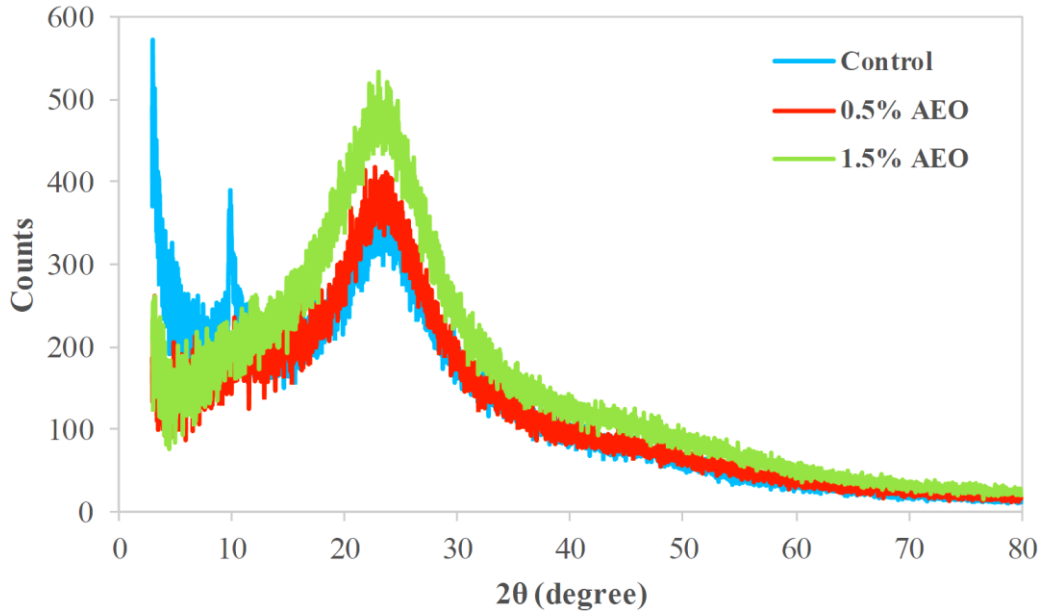

Fig 2. XRD patterns of control and FG/CMC/PG ternary films containing different amounts of AEO

3.8. Differential Scanning Calorimetry (DSC)

Polymer miscibility is an important factor in enhancing the performance of new materials derived from polymer blends and significantly impacts their final properties [48]. The DSC test was performed to investigate the thermal properties, further understand the structure, and examine the interactions between the polymer solutions prepared based on FG, CMC, and PG. The melting temperature (Tm) and glass transition temperature (Tg) are related to the crystallinity of the film samples. At a lower glass transition temperature, the films are hard and brittle, while at a higher glass transition temperature, the films exhibit more flexibility [34]. The melting temperature is also dependent on the molecular motion of polymer chains and the destruction of their regular or aggregated molecular structure, which significantly influences the properties and applications of the materials [49]. The thermal indices obtained from the DSC thermograms of the three-component FG/CMC/PG films containing various AEO ratios (0.5% and 1.5%) in the temperature range of 25 to 400°C are shown in Figure (3). Two endothermic peaks were observed for all films; the first endothermic peak occurred in the temperature range of 83.3°C to 184.7°C, which may be related to various phenomena such as moisture evaporation from the sample, degradation of the plasticizer (glycerol) and emulsifier, and helix-coil transition of gelatin [50]. Additionally, this could be attributed to the intense endothermic melting transition at 184.7°C, with an enthalpy of 133 J/g [51]. Meanwhile, the second peak in the temperature range of 237.3°C to 255.6°C indicates thermal degradation (Td) due to the breakdown of the carbonyl groups in the side chains of CMC, thermal degradation of peptide bonds in the gelatin backbone, pyrolytic decomposition of the polysaccharide backbone, as well as the chemical degradation of AEO [52, 53]. As shown in Figure (3), the addition of AEO resulted in an increase in the melting temperature (Tm) of the three-component film from 245.7°C to 255.6°C. As we know, the melting phenomenon is caused by the increased kinetic energy of molecular thermal motion as the

temperature rises [35]. Here, with the addition of AEO, a higher temperature is required for the thermal motion of the molecules. The obtained results are in line with the findings reported by He et al. [35] regarding the addition of antioxidant extract from bamboo leaves to a carboxymethylcellulose/gelatin fish film matrix.

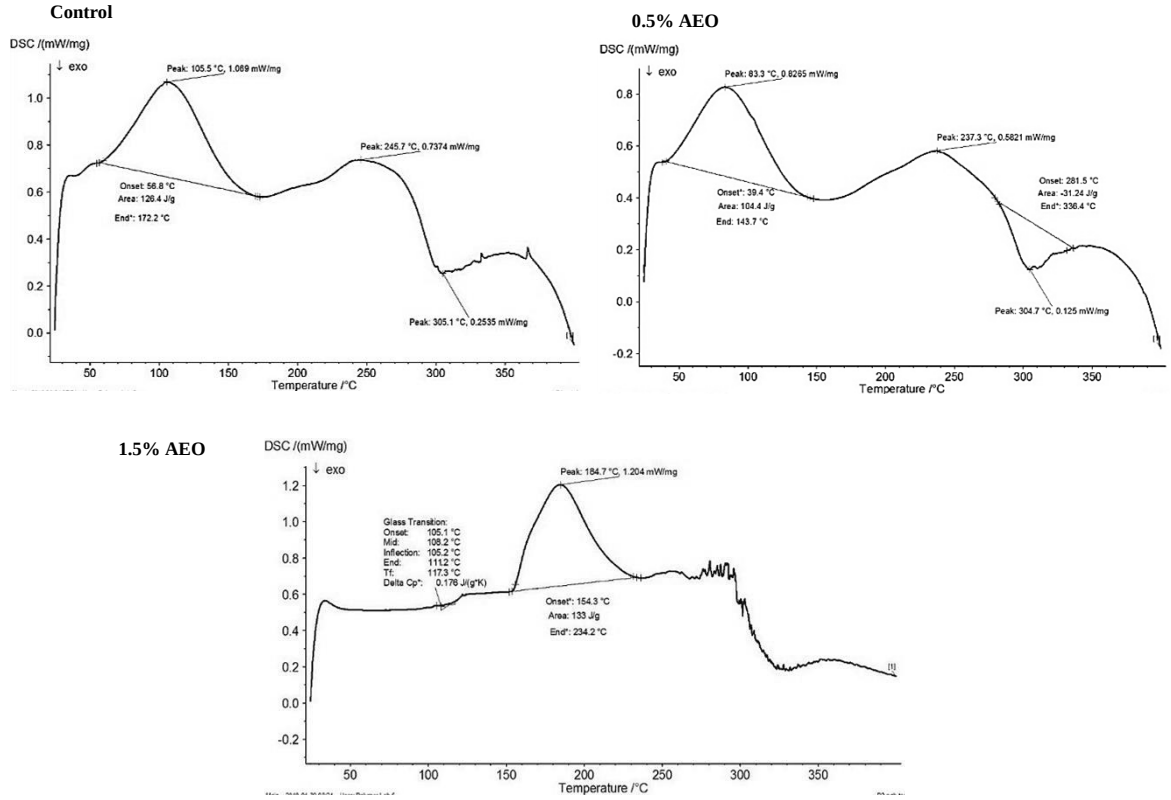

Fig. 3 DSC thermograms of control and FG/CMC/PG ternary films containing different amounts of AEO

3.9. Scanning Electron Microscopy (SEM)

Studying the microstructure of the film matrix helps in better understanding the mechanisms of water vapor transmission, as well as its mechanical and optical properties. In Figure (4), SEM images of the surface and cross-section of the ternary control film and the ternary films containing various concentrations of AEO are presented. As shown in Figure (4), the ternary control film (FG/CMC/PG) exhibits a continuous, smooth, homogeneous microstructure without any pores or cracks, indicating the compatibility of the three polymers. The addition of AEO up to a level of 0.5% had no effect on the surface morphology of the ternary film, indicating that AEO was uniformly and well dispersed within the film matrix. However, with the addition of 1.5% AEO to the film matrix, some circular structures were observed on the film surface, which may have formed due to the movement of excess AEO droplets to the surface during the film drying process [50]. This phenomenon may also be attributed to the aggregation of hydrophobic oil droplets during the film formation process [26]. Moreover, the cross-sectional images of the ternary control film and the AEO-containing films revealed a continuous and compact morphology without irregularities such as air bubbles or pores, and with no evidence of phase separation, as would be expected for a homogeneous material [45].

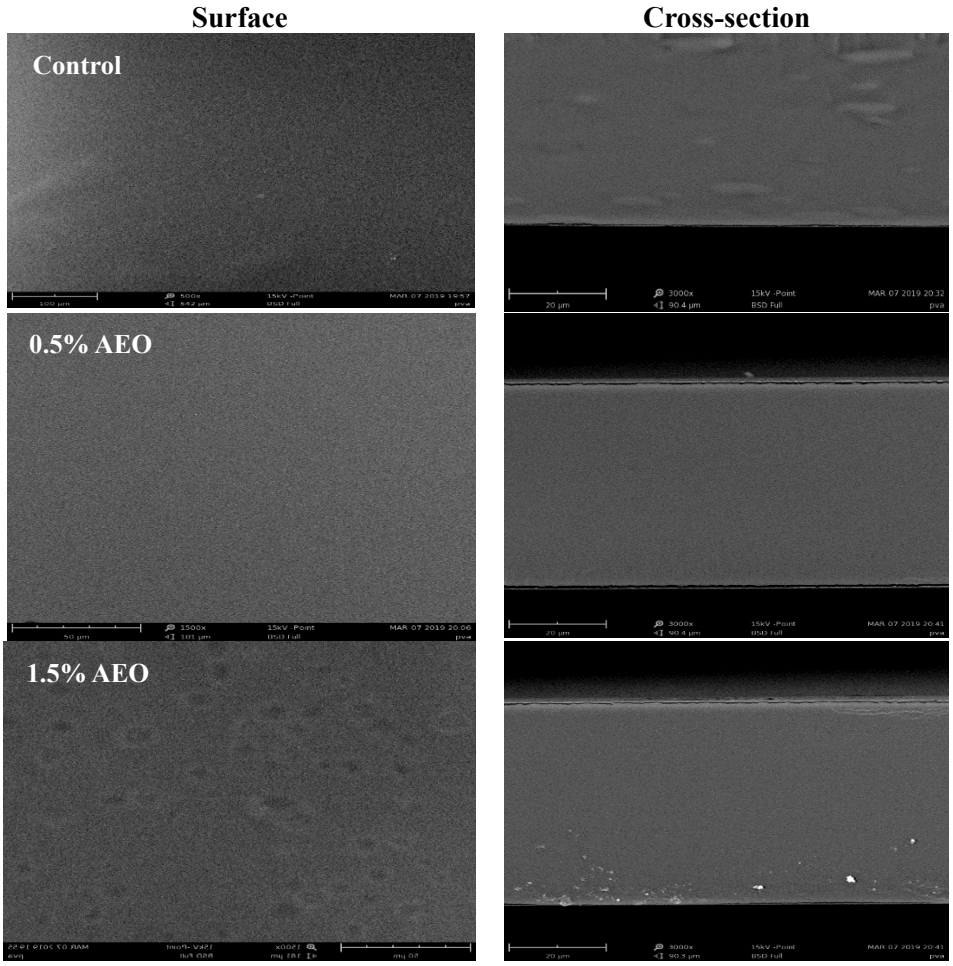

Fig. 4 SEM images of control and FG/CMC/PG ternary films containing different amounts of AEO

3.10. Antibacterial Efficiency of the Ternary Film Containing AEO

One of the most important requirements of advanced packaging materials is to inhibit or prevent bacterial growth and colonization on the surface of food products [5]. Plant essential oils are among the promising molecules for the development of antimicrobial food packaging materials, as they have demonstrated effective performance against harmful Gram-positive and Gram-negative bacteria [54]. Therefore, in the final stage of the present study, the antimicrobial activity of the ternary FG/CMC/PG film containing three different concentrations of Artemisia Essentia Oil (AEO) was evaluated against Gram-positive bacteria *Staphylococcus aureus* and *Listeria monocytogenes*, as well as Gram-negative bacteria *Salmonella enteritidis* and *Escherichia coli*. As shown in Table (3), the ternary control film exhibited no antibacterial activity against the tested microorganisms. The absence of an inhibition zone can be attributed to the inability of the polymer components to inhibit bacterial growth. Haghighi et al. [40] also reported that a gelatin/chitosan composite film exhibited no antimicrobial activity against four major foodborne bacterial pathogens, including *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella typhimurium*. These researchers attributed the lack of inhibitory effect to the limited diffusion of chitosan in the agar medium or the inability of gelatin to suppress bacterial growth. As shown in Table (3), the addition of AEO to the ternary

films resulted in the formation of growth inhibition zones (dependent on the essential oil concentration) against all four bacterial strains ($P < 0.05$). The ternary film containing the highest concentration of AEO (i.e., 1.5%) effectively inhibited the growth of the tested microorganisms, producing inhibition zones ranging from 7.7 to 9.5 mm ($P < 0.05$). Oxygenated monoterpenes such as 1,8-cineole, camphor, and terpinen-4-ol, which are the characteristic components of AEO, have been reported to exhibit antimicrobial activity [55]. Hashemi and Khodai [56] reported that edible films made from basil seed gum containing Artemisia sieberi essential oil exhibited antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, and *Pseudomonas aeruginosa*. These researchers attributed the antimicrobial activity of Artemisia essential oil to the synergistic effects among its main components, including camphor, isoborneol, 1,8-cineole, caryophyllene oxide, β-eudesmol, and terpinen-4-ol. As Table (3) shows, the diameter of the inhibition zones against Gram-positive bacteria is slightly larger than that against Gram-negative bacteria, likely due to the presence of an outer membrane surrounding the cell wall of Gram-negative bacteria, which limits the diffusion of hydrophobic compounds through its lipopolysaccharide layer [54]. These results indicate that the FG/CMC/PG ternary film containing AEO could be a promising antimicrobial packaging material for combating foodborne illnesses.

Table 3 Antimicrobial activity of FG/CMC/PG ternary films containing different amounts of AEO

Inhibition zone (mm)				
Sample	**S.aureus**	**L. monocytogenes**	**S. enteritidis**	**E. coli**
Control	0.00^{d}	0.00^{d}	0.00^{d}	0.00^{d}
0.5% AEO	4.32 ± 0.21^{c}	4.80 ± 0.45^{c}	3.98 ± 0.47^{c}	3.67 ± 0.31^{c}
1% AEO	5.97 ± 0.38^{b}	6.17 ± 0.55^{b}	5.42 ± 0.41^{b}	5.20 ± 0.28^{b}
1.5% AEO	8.52 ± 0.46^{a}	9.50 ± 0.63^{a}	8.01 ± 0.85^{a}	7.71 ± 0.40^{a}

4. Conclusions

Amid global resource scarcity, severe environmental pollution, and significant food waste, the rapid development and adoption of environmentally friendly antimicrobial packaging materials with preservation capabilities and non-toxic properties is essential for advancing sustainable development. This study aimed to evaluate the impact of *Artemisia* (AEO) essential oil on the physicomechanical, microstructural, and antimicrobial properties of ternary FG/CMC/PG films. The results showed that the microstructure of the film, thermal transition parameters including melting temperature (Tm) and degradation temperature (Td), tensile strength, elongation at break, water vapor permeability, water solubility, water absorption, color and opacity, as well as the antibacterial properties of the ternary films were affected by the incorporation of *Artemisia* (AEO) essential oil. Overall, the evaluation of the film properties indicated that *Artemisia* essential oil can enhance certain functional characteristics of biopolymer-based materials, improving their desirable properties for potential application as eco-friendly food packaging. Based on the results obtained, the antimicrobial films developed in this study could be used in the design of multilayer packaging materials (as an

inner layer in direct contact with food) to help extend the shelf life of packaged food products.

Conflict of Interest

The authors declared no conflict of interest.

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مقاله پژوهشی

ارزیابی اثرات اسانس درمنه خزری (*Artemisia annua L*) بر خواص فیزیکومکانیکی، ساختاری و ضدمیکروبی فیلم خوراکی بر پایه ژلاتین ماهی

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چکیده

در مطالعه حاضر، فیلم ضدمیکروبی جدید بر پایه ژلاتین ماهی (FG)/کربوکسی متیل سلولز (CMC)/ صمغ فارسی (PG) حاوی اسانس درمنه خزری (AEO) در سه سطح مختلف (۰/۵، ۱ و ۱/۵٪، وزنی/حجمی) از طریق روش قالب‌گیری ساخته شد و ویژگی‌های فیزیکومکانیکی، ساختاری و ضدمیکروبی فیلم‌های حاصله مورد ارزیابی قرار گرفت. نتایج نشان داد با افزایش محتوای AEO، WVP فیلم‌ها که در محدوده ۰/۴-۷۴۶/۵۱ g mm/kPa h m^2 بود و نیز حلالیت (FS) فیلم‌ها افزایش یافت، درحالی‌که میزان جذب آب (SR) کاهش یافت. نتایج سنجش ویژگی‌های مکانیکی نشان داد که ادغام AEO باعث افزایش معنی‌دار انعطاف‌پذیری (EAB) فیلم‌ها ($P<0/05$) و کاهش استحکام کششی (TS) شد. طیف‌سنجی FT-IR تشکیل پیوندهای هیدروژنی بین‌مولکولی را بین گروه‌های عاملی اجزای پلیمری و AEO نشان داد که به نوبه خود منجر به بهبود پایداری حرارتی و خواص مکانیکی فیلم‌های سه‌جزئی گردید. تجزیه‌وتحلیل پراش اشعه ایکس (XRD) نیز امتزاج‌پذیری بین اجزای تشکیل‌دهنده فیلم را نشان داد. تصویربرداری میکروسکوپ الکترونی روبشی (SEM) مؤید آن بود که فیلم‌های سه‌جزئی حاوی AEO دارای ریزساختار پیوسته، صاف و همگن هستند که نشان‌دهنده سازگاری بین اجزا می‌باشد. به‌علاوه فیلم‌های سه‌جزئی حاوی بیشترین غلظت AEO (۱/۵ ٪) فعالیت ضدباکتریایی قابل قبولی را نسبت به باکتری‌های گرم مثبت استافیلوکوکوس اورئوس و لیستریا مونوسیتوژنز و باکتری‌های گرم منفی سالمونلا انتریتیدیس و اشرشیاکلی نشان دادند. یافته‌های این مطالعه نشان می‌دهد فیلم‌های سه‌جزئی FG/CMC/PG حاوی AEO می‌توانند به عنوان مواد بسته‌بندی امیدوارکننده برای محصولات غذایی استفاده شوند.

واژه‌های کلیدی: فیلم‌های ضدمیکروبی، ژلاتین ماهی، کربوکسی متیل سلولز، صمغ فارسی، اسانس درمنه خزری.

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